PARR

A Study of the Influence of Ether,

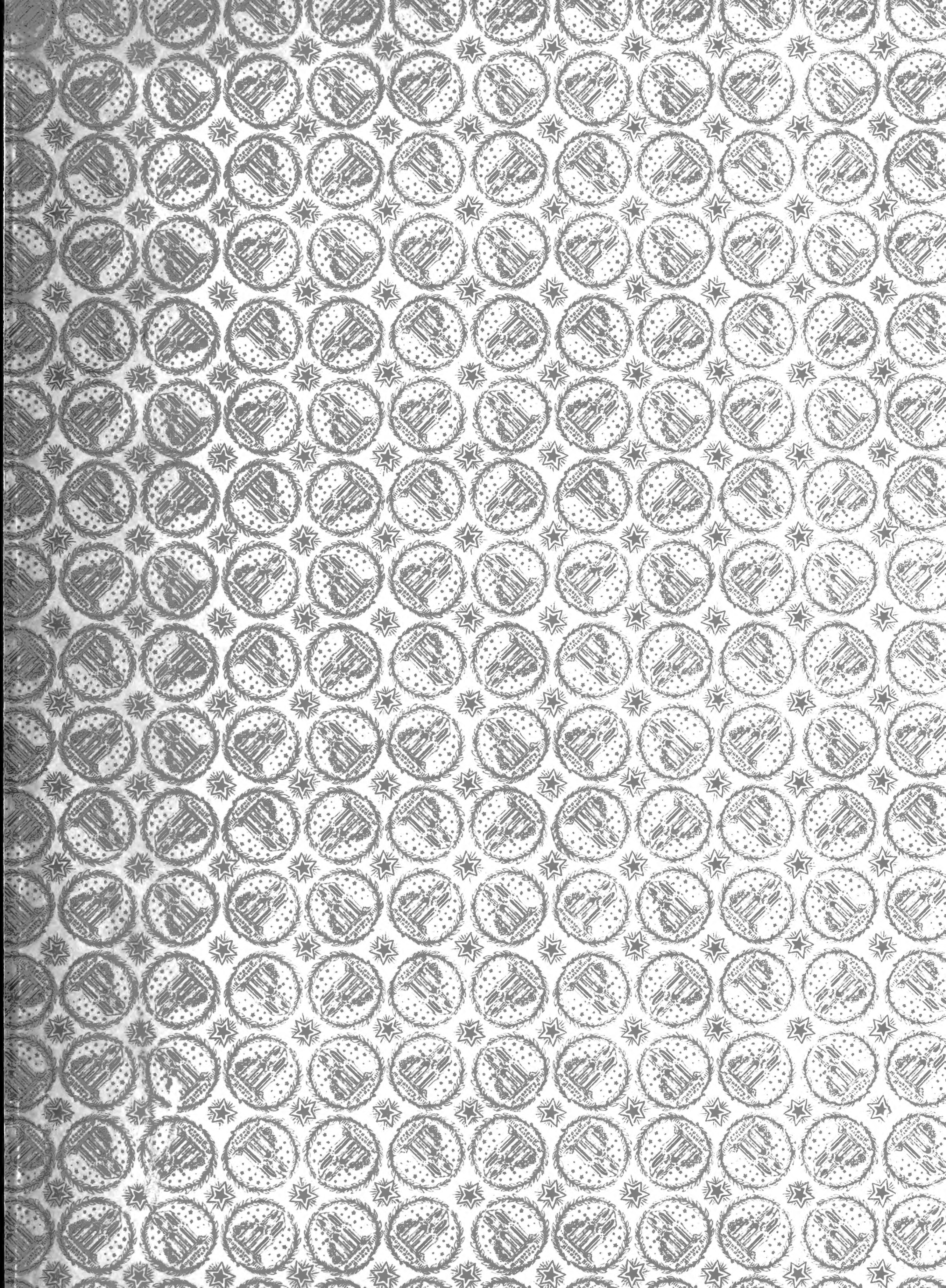
and of Calcium and

Magnesium during Inanition

Botany

A. M.

1911


Monastery Hill

A STUDY OF THE INFLUENCE OF ETHER, AND OF CALCIUM AND MAGNESIUM DURING INANITION

BY

ROSALIE MARY PARR
A. B. University of Illinois, 1906

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF ARTS

IN BOTANY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1911

1911
P24

UNIVERSITY OF ILLINOIS

THE GRADUATE SCHOOL

June 1, 1911

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Rosalie Mary Parr

ENTITLED A Study of the Effect upon the Cell of Ether, and of Magnesium and Calcium, During Inanition.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Arts

Chas. F. Hottes
In Charge of Major Work

T. J. Burrill
Head of Department

Recommendation concurred in:

Committee on Final Examination

A STUDY OF THE EFFECT UPON THE CELL OF ETHER, AND OF MAGNESIUM AND CALCIUM, DURING INANITION.

Means of discovering the processes of plant activity are limited. If a process may be held in check for a time, or if a stimulus cause it to act with greater emphasis, we may bring it partially under our control by changing the factors so affecting it, and in this way gain some knowledge of its working.

In the gradual decline of the life processes in starvation, we find an opportunity to hold apart and to analyze physiological conditions in a manner not possible when the cells are well-fed and growing normally. Since here the supply of food is a limiting factor an interesting subject of study is presented in the changes and the translocation of food materials. In his study of "inanition" Professor Hottes has shown that a seedling deprived of reserve food substances makes use of material within the older root cells to continue the growth of the embryonic parts. A seedling of Vicia having the cotyledons removed and kept in the dark, while other conditions are favorable to growth, develops new cells and increases in length twenty to thirty millimeters by the transportation of the plastic substances within the cells to the meristematic regions.

Zalaski[9] has given proof in his study of "the action of ether upon the transformation of the albumen in etiolated embryos" that ether stimulates the movement of albumen, causes diasmosis of carbohydrates and of albumen, and brings about a regeneration of albumen within the plant cells.

Loew[4] finds in his study of "the effects of lime and magnesia upon plant growth" that magnesia serves as a vehicle for the transportation of phosphoric acid, sulphur, and nitrogen, and thus facilitates albumen formation, while calcium prevents an excess of magnesium or of oxalic acid and is necessary in the formation of nucleo-proteids. When both calcium and magnesium are present in well balanced proportions growth is aided very materially.

In view of these facts my purpose is to find the effect upon the cell of magnesium and calcium, and of ether, in the root-tips of Vicia faba during inanition.

In order to determine the effect of these reagents it has been necessary to make a careful study of the normal root tip, and also of the changes which take place during the process of what may be called "normal starvation".

Methods

Seeds of Vicia faba were soaked in water over night. The next morning they were planted in moist sphagnum and left to germinate. They were examined each day for mould, and, if necessary, were removed, washed in water, and replaced in the sphagnum which had been sterilized in boiling water.

Seedlings from two to six centimeters in length were selected and the cotyledons carefully removed with a sharp scalpel. The rootlets were measured and placed through numbered bits of paper and through cotton net, allowing them to extend into the

solutions contained in earthen liter jars. They were covered with similar jars to exclude the light and kept at as nearly a constant temperature as possible.

Measurements of growth were made from day to day, and specimens were taken from the jars at stated intervals and fixed in a solution composed of nine grams of corrosive sublimate, nine cubic centimeters glacial acetic acid in three hundred cubic centimeters of seventy percent alcohol. The stain used was Haidenhain's Iron-haemotoxalyn.

One tenth normal solutions of calcium and magnesium nitrates, and chlorides, and a saturated solution of ether were made up as stock solutions, and from these, solutions which contained varying quantities as will be designated in the description of the several experiments, were made as required.

Normal Tip

A study of the normal tip was made and carefully compared with the observations of Rosen[7] and of Hof[3] upon the root tip of Vicia faba.

The formative region of the normal tip is composed of compact, meristematic cells, many of which are in the process of division. The resting cell of this region is filled with foam-like cytoplasm in which a nucleus occupying about one-third of the cell space is embedded. Within the nucleus are one or two nucleoli each surrounded by a clear space, or "hof". One vacuole may usually be seen in a nucleolus, though three or four are some-

times evident.

In the dividing cell the nucleus undergoes a series of transformations. The chromatin bodies increase in size and arrange themselves along a linin thread, like a string of beads. This chromatin-linin strand gradually flattens out and divides lengthwise so that a clear space may be seen through much of its length. The two halves then become segmented into chromosomes. During the spireme stage the nuclear membrane remains unbroken and two rather dense cone-like cytoplasmic masses appear at opposite ends, or poles, of the nucleus forming the "anlage" of the mitotic spindle. These cones develop into a mass of delicate threads, focussing to two single points, bipolar, or to several points at one or both ends, multipolar, and extending entirely across and around the nucleus reaching from pole to pole, or connecting the segmented chromosomes with the poles. The chromosomes have a J form and arrange themselves for the nuclear plate after which they are drawn toward the poles where they form the diaster. A cell plate forms between the daughter nuclei and the spindle threads disappear while the chromosomes re-combine into a second skein stage. A nucleolus soon forms in each of the daughter nuclei and the cell passes into the resting stage.

As the cells become older they become longer and broader by deposition of material in the walls. Vacuoles appear in the cytoplasm. The nucleolus divides into several nucleoli which gradually become smaller. The nuclei of the xylem region are large and spherical excepting those of cells forming tracheids which lengthen and decrease in size. The nuclei of the bast

region elongate somewhat and contain many nucleoli.

Rosen[7] believes that where two nucleoli lie together it is hard to prove that these are not fusion forms. He also states that the nucleus fated to divide shows only one nucleolus. The fact that most of the older cells show several nucleoli would contradict the view that two nucleoli in a cell must necessarily fuse. Otherwise the younger cells would contain several, and the older cells but one nucleolus. In several cases I have found nuclei in the early prophase containing two nucleoli, which shows that fusion of the nucleoli is not necessary before division takes place.

The cells of the cambium retain their ability to divide and thus increase the size of the central cylinder by the production of secondary tissue. The resting nucleus of the differentiating region is composed of a compact linin network containing chromatin bodies. As the embryonic character is lost the chromatin bodies become less evident, and the linin substance becomes more prominent. In the cells in the process of forming tracheal vessels, the nuclei disappear as the walls become differentiated and the end walls absorbed.

Normally Starved Tip

My experiments upon the normally starved tip are a repetition of those already performed by Professor Hottes. The results obtained agree with his in most essentials.

The root-tips were placed in tap-water and kept in the dark at a fairly uniform temperature. Specimens were taken each

third day until death ensued which usually took place after twenty days. Daily measurements of the tips show that growth continued until the fourth day, after which there was little if any increase in length. Swellings due to the rudiments of lateral roots appeared at irregular intervals along the root to within a centimeter of the tip. The initial length of tips used by Professor Hottes was about three centimeters and the maximum increase in length was two centimeters. My tips varied in their initial length some of them measuring six centimeters, and a growth of three centimeters occasionally took place in these. Since the longer roots furnish more food material which may be transported the growth is correspondingly longer.

Microscopic examination of specimens taken after three days of starvation shows that the region of elongation has in part replaced the formative region, and hence, the latter is reduced in extent. The amount of cytoplasm in all cells of the tip is less than in the normal. The cytoplasm has lost its foamy consistency in the formative region and the cells contain many vacuoles. In the stele the vacuoles occupy the greater amount of cell space, the cytoplasm having largely receded to the cell walls. Extruded chromatin bodies are visible in many of the cells, but I failed to find the protein bodies described by Professor Hottes, my failure probably being due to the method of fixing and staining.

The resting nuclei of the formative region are somewhat smaller, but the chromatin content about the same as in normal cells. In the stele the resting nuclei are flattened and pressed to the walls. Nucleoli are smaller than in normal cells, and

vacuoles in them are rare. In the cells of epidermal regions and in the older cells of the stele several nucleoli are found in a single nucleus. Mitotic figures, slightly reduced in size, appear in the formative region and in the cambial strands as in normal tips.

After six days the cytoplasm is much reduced in quantity. The nuclei of the formative region are in the corners of cells, and those of the stele are flattened against the lateral cell walls. Nucleoli have increased in number but decreased in size. Mitotic figures have entirely disappeared from the meristematic regions.

General Observations

A comparison of seedlings grown in the different solutions shows some peculiarities of external structure. Seedlings kept in tap water show very slight swellings due to rudimentary lateral roots within a centimeter of the tip. In seedlings of the calcium- magnesium solution the rudimentary roots are less prominent and farther removed from the tip. In the ether solution rudimentary roots become much more prominent and seem somewhat nearer the tip than those in tap water.

The general effect of ether, and of calcium and magnesium upon the growth of the roots is shown in the following table. The sum total of the initial lengths, and the sum total of increase in length of all roots grown respectively in tap water, in .4 percent ether solutions, and in solutions containing .8 percent magnesium chloride and 1 percent calcium chloride are here expressed

in millimeters. The seedlings in ether solution show a decrease in the percentage of growth while those under calcium-magnesium influence show a considerably larger amount of growth than those kept in tap water.

Table I.

Table comparing the percentage of the total increase in the length of roots in tap water, in .4% ether solution, and in .07% $MgCl_2$ + .11% $CaCl_2$ solution.

Solution	Number of Roots	Total Initial Length	Total Increase in Length	Total Percentage of Growth
Tap Water	85	2690 mm.	1303 mm.	48.4 %
.4% Ether	29	1137 mm.	495 mm.	43.3 %
.07% $MgCl_2$ + .11 % $CaCl_2$	51	1848 mm.	1055 mm.	56.5 %

A study of the relation of the initial lengths of roots to the total amount of growth leads to the conclusion that while the amount of material suitable for translocation is greater in older roots the percentage of such material is less than in the younger tips.

In Table II the roots used in all experiments on normal starvation are classified in groups according to initial length.

Group A includes all tips from fifteen to thirty millimeters, group B all from thirty to forty millimeters, and group C all from forty to sixty millimeters.

Table II

Showing relation of initial lengths of roots to total amount and percentage of growth.

Group	Included Lengths of Roots	Number Seedlings Measured	Total Initial Length	Average Initial Length	Total Increase in Length
A	15-30 mm.	46	967 mm.	21. mm.	565 mm.
B	30-40 mm.	12	415 mm.	34.5 mm.	204 mm.
C	40-60 mm.	27	2690 mm.	44.8 mm.	1303 mm.

(con.)

Group	Average Increase In Length	Percentage Increase in Length
A	12.2 mm.	58 %
B	17. mm.	49 %
C	19.2 mm.	40 %

Effects of Ether

Seedlings of Vicia placed in solutions of three-tenths, four-tenths, and five-tenths percent concentration respectively gave so nearly the same results that it is not necessary to describe the experiments here separately.

Microscopic study of the sections of tips from ether solution killed the third day show a more vacuolate condition of the cytoplasm than those exposed to inanition alone. The cytoplasm seems more coarsely granular, and quite frequently may be seen collected about the nucleus giving it a ragged appearance.

The elongating cells of the plerome extend almost through the formative region, reaching nearer the tip than in normal starvation. Rows of small empty cells, and rows containing diminutive nuclei appear just inside the cambial strands, while in the central rows of pith cells the nuclei seem abnormally large and stain very lightly. Deeply staining bars across the narrow xylem cells are probably composed of dead nuclei under pressure. The elongated nuclei in the cells near these have peculiarly distributed chromatin.

The cells of the tip are smaller than in normally starved roots, and very few mitotic figures are evident in the formative region. The mitotic figures of the cambial strands and in the rudiments of secondary roots are more nearly normal than are those of the formative region of the same tip. The cytoplasm is stained in some cells as though chromatin substance were distributed through it and considerable extruded nuclear substance appears as globules

in the cytoplasm and in the vacuoles of the cells.

Many nuclei are irregular in shape with the chromatin collected in masses. In the cells surrounding the small formative region the increased number of nucleoli give the same appearance found in the sixth day of normal starvation. In the older cells of the plerome many of the nucleoli are elongated and some are dividing. The nucleoli diminish in size much more rapidly in the older cells than in those of normally starved roots and many seem to be extruded into the cytoplasm. Vacuoles are rare in the nucleoli within the nuclei but are often evident in those of the cytoplasm.

Two nuclei may be found in a cell, or occasionally two daughter nuclei in the second spireme stage may be seen with no cell wall between them. In certain cells in the diaster stage of division small bodies resembling nucleoli may be seen where the cell plate is forming. In one case these bodies are fused giving the appearance of the elongated nucleoli of older cells. In one cell a spherical body appears above and one below the nucleus with slight connecting fibres between the nucleus and one of the bodies. This is probably a condition resulting from arrested division.

Tips taken from the ether solution the ninth day show a condition similar to that found in normal starvation in the same length of time. The cells contain but little cytoplasm, the nuclei are smaller and pressed to the walls. The nucleoli are elongate and small in most cases. Cell walls are quite sinuous as a result of a loss of turgidity. The tracheal vessels contain

globules which probably are substances precipitated by the fixing agents. The cells of the formative region have expanded and do not seem crowded excepting in the extreme tip.

The duration of life does not seem different from that in normal starvation. I find the limit in both to be about twenty days.

Discussion

In the experiments in which starvation in ether solution is proceeding we have a physiological action which in many respects is a combination of the effects produced where each is taken alone.

The cells under the mild ether influence have increased in number and decreased in size. The completion of division of cells already in a dividing state has been hastened. In inanition alone the third day shows mitosis progressing in almost a normal condition. In ether solution the starving cells have completed division by the third day with few exceptions. While ether has acted as a stimulus hastening the mitotic process, the lack of food substance has interfered with normal cell development in quantity of cytoplasm and of nucleoplasm. Differentiation seems to have taken place normally. We thus find the same number of cells which would have been normally present in a mitotic condition the third day, in a resting and differentiating condition, and dwarfed by the lack of nourishment. This seems an exception to the rule of Eugen Schultz, namely, that increase and decrease in the size of an organism is a function of cell division and not a function of cell size.

This rule stated differently implies that lessening the size of an organism lessens the number and not the size of the cells contained within the organism. In the starved roots from the ether solution the number of cells remains practically constant to the number under normal conditions while the size of the cells decreases as the size of the root decreases.

Although an occasional mitotic figure is present in the specimens killed the fifth and sixth days, cell division is not sufficiently active to account for the growth of one or two centimeters made within the first five days of starvation. That the lengthening is not due to further cell division may be determined not only by the few mitotic figures present, but by the replacement of a large part of the formative region by the region of elongation which comes within a few cell rows of the extreme tip.

Buscalioni[1] must have observed something of this which led to his statement that "amitosis leads to tissue-building". The continued lengthening of the root in the absence of mitosis agrees in one phase with the observations of Buscalioni. The growth takes place in the absence of mitotic figures. However, the rare cases where there seems a possibility of direct division taking place, as two nuclei separated by a cell plate and no spindle fibres present, may be explained by the fact that in the gradual decline of the life processes, normal cell division is arrested in its progress, and that one of the first visible evidences of its arrest is the interference with the normal functioning of the spindle fibres. This leaves the newly formed daughter nuclei to round off and complete their formation while the cell plate is

left without the means of obtaining the necessary substance to complete the construction of the cell wall: These cases of two nucleated cells are so rare that the lengthening of the root cannot be accounted for by means of amitosis, even did it seem otherwise plausible.

The formative region is perceptibly smaller due to the elongation of cells and to the lack of dividing cells. While our limiting factors interfere with further mitosis, they do not prevent the deposition of material in the walls of fully formed cells. This material, doubtless, is furnished from the cytoplasm and the nucleus as in normal growth. The diminution in size of the nuclei as the cells grow older, and their extreme minuteness in various rows of cells would give evidence that a heavy draft for food-materials is made upon them. This would indicate that the lengthening of the root is due to the transformation of these materials into cell walls.

While this elongation has continued for the same relative length of time as in normal starvation, the total increase in length is less, as may be seen in the preceding table. This result is in accordance with Gerassimow's[2] statement that etherization makes the cells physiologically weaker.

Pfeffer [6] believes that the poisonous action of ether is due to loose chemical union with the protoplasm. It is probable that this combination takes place with the ectoplast producing a membrane of greater permeability to the diffusable molecules within the cell, and at the same time a partial precipitation of substances

in the cell sap may take place resulting in a decrease in turgor and finally producing plasmolysis. Since cell division only takes place in turgid cells a cessation of mitosis follows. If this action is reversible, a slight change of temperature, or other conditions, would permit the cell substances to return to a normal state and mitosis would be resumed, as has been found by Pfeffer [6], Nathansohn [5], and others.

Ether seems to emphasize the effect of starvation upon the nucleoli; or perhaps, hastens the metabolic or chemical action going on within them. By the third day of subjection to ether influence the nucleoli have increased in number far more than under normal starvation in the same length of time. They are irregular in shape, many are flattened, and some are in the budding condition. The quantity of nucleolar substance, however, seems less than in normal starvation. The derivatives of the nucleolar substance may be more diffusable under the ether influence, and the chemical reaction causing its decomposition may thus be kept up a greater length of time. This may be true whether these derivatives are used in tissue building or are cast off as waste material. The decrease in the size takes place both in the nucleoli of the nucleus and of the cytoplasm. A decrease in the number and size of the nucleoli seems to precede the decrease in the size of the nuclei, - an indication that both are used to supply material for tissue-building.

An argument in favor of considering the action of ether as chemical combination and not catalytic action nor physical absorption may be found in the fact that mitosis is affected to a

greater degree in the formative region than in the cambial strands and in the rudiments of secondary roots. Evidently chemical union is taking place nearest the region of absorption. The increased size of the external swellings due to the rudiments of the secondary roots also shows that growth is less affected than in the tip. Were the ether merely a catalytic agent we should expect that its activity would not be limited to the area nearest its place of absorption, and if it were a case of physical absorption the simple laws of diffusion in solution would carry it equally to all parts.

In Zalaski's [9] study of the action of ether upon the transformation of albumen in etiolated seedlings, he found that more albumen moved from the cotyledons into the axis than under ordinary conditions. His experiments show that either there is less breaking down of albumen while ether causes its strong diffusion through the membranes, or that the albumen broken up into simpler compounds for transportation is rapidly regenerated in the plant cell under the influence of ether. In his experiments with lupines regeneration could be proven only in very young embryos, but he obtained quite convincing results with wheat seedlings. Since chromatin is a proteid substance, a greater or less amount of chromatin may indicate a like increase or decrease in the albumen content of the cell. While it is difficult to determine the relative quantity of chromatin by the method of staining and it is practically impossible to decide by the color intensity the chromatin content in various slides, I believe that the relative sizes of nuclei will give a fair estimate in regard to the amount of chromatin in the

etherized seedlings, compared to those of normal conditions. That the nuclei are much smaller and consequently the chromatin content much less is quite evident. I, therefore, conclude that in seedlings of the size used, and with the solutions of the strengths used in these experiments albumen regeneration does not take place.

On the other hand, plasmolysis of the cells shows that exosmotic action is increased by the ether solution, confirming Zalaski's statement, namely, that ether causes strong osmosis of albumen and of carbo-hydrates in the cell.

Effects of Calcium and Magnesium

Several experiments were tried using the several percentages of calcium and magnesium salts in solutions that were found most advantageous to growth by May[12] in his experiments with lupines and with wheat seedlings. The solutions which gave the best results with Vicia faba contained eight hundredths of a gram-molecule of magnesium chloride and one-tenth of a gram-molecule of calcium chloride made up in tap water to one liter. Under the most favorable conditions the increase in growth of seedlings in this solution was quite remarkable. As may be seen in Table I, the increase in length of normally starved roots is forty-eight and four-tenths percent while that of seedlings in the magnesium-calcium solution is fifty-six and five-tenths percent of the initial length. Measurements of roots grown in this solution show that they not only exceed in length those of the tap water and of the other solu-

tions, but growth continues for at least a day after it has ceased in the roots of the other solutions. In contrast to the effect of the ether solution which weakens the cells physiologically the magnesium-calcium solution evidently strengthens them.

Though not so small as in the formative region of the tips grown in ether solution, the size of the cells in the tips of the magnesium-calcium solution is less than in normal seedlings, and the region of elongation extends almost through the formative region.

Microscopic examination of the formative region of these seedlings fixed the third day, reveals a condition similar to that found in well-fed plants grown under favorable conditions. The cytoplasm is a trifle more vacuolate. Where vacuoles occur they are small, and several surround the nucleus which is large and full and located in the center of the cell. The proportion of resting nuclei seems large and many of them contain two nucleoli of the size found in normal cells. The seeming anomaly of the smaller percentage of dividing nuclei, while increase in length continues beyond that of normally starved tips, may be explained by the fact that the translocation of materials has been facilitated and this has made possible a more rapid construction of nucleo-proteids necessary for nuclear growth and division in the formative region, and thus the supply from the older cells of the root is soon exhausted. However, more material available for tissue formation is now in the cells of this region than is present in the same length of time in normal starvation. Doubtless, the presence of magnesium

keeps the movable materials still in a condition for nuclear and cytoplasmic construction after the supply is too limited to keep up the process of division. Thus the period of elongation is increased and the growth of the root continues longer than in the process of normal starvation. Large nuclei of the lower pith cells stain deeply in contrast to cells of the same region from ether solutions. This indicates a greater amount of chromatin present showing the fact of increased food translocation for its formation.

Nuclei in a dividing state show thick chromosomes. The mitotic spindle is clearly defined and is distinctly bi-polar with few exceptions while cell-plates form normally.

Many cells contain two nucleoli equal in size to those of similar cells in the normal tip. The number of small nucleoli derived from the fundamental nucleolus found in older cells are as few in number as in normal cells.

Many large nucleoli may be found outside the nucleus. These all appear on the same side of the nuclei indicating that they were taken out by the knife; but this fact in itself shows them to be of a firmer consistency, since this condition was not found to this extent in the normal, in the normally starved, or in the tips of the ether solution.

This denser form of nucleoli retain their globular form, remain in larger vessels, show less "budding", and do not disappear in older cells. The varying consistency of the nucleoli may also be seen in the tips from ether solutions by the fact that they are

less dense as shown by elongation, and by the formation of a greater number of small nucleoli which disappear as the cell grows older. This variation in density of the nucleolus may serve to throw light upon the food relations of the nucleoli. The cells from well-fed roots and from those in magnesium calcium solutions which have a good supply of food substance in the tips show the denser masses of nucleolar substance, while the normally starved and those from ether solution having less available food substance, show the nucleoli less dense in nature, more divided, and as Professor Hottes has shown, less in mass. This gives an argument in support of Paratore's [10] view that the nucleolus serves as the metabolic center of the nucleus.

Blackman [11] considers the plant cell a colloidal honey comb, in which case this action of the nucleolus furnishes an example of a colloidal solution of food materials in the nucleolar substance. That the action is a physical process rather than a chemical combination may be argued from the fact that the fundamental, or parent, nucleus does not entirely disappear under any of the experimental conditions here given, although it continues to becomes smaller until the death of the cell, the rate of its decrease depending upon the character of the active agents in the solutions.

In contrast to the formative region the older parts of the roots give more evidence of inanition than do those subjected to starvation in tap water in the same time. The cytoplasm of these cells is almost exhausted. Many nuclei are small with the

chromatin unevenly distributed, and some have evaginations in the nuclear membrane as though beginning to disintegrate. Many nuclei of the periblem have a position on the lower side of the cell instead of being pressed to the lateral walls as in normal starvation. All of these observations lead to the conclusion that materials are transported from the older cells to those in the embyronic condition more completely, and more rapidly, under the influence of the calcium and magnesium solution than occurs under conditions of normal starvation.

In the experiments described in the preceding pages the constant limiting factor has been the small supply of food material available to the growing seedling. In the gradual downfall of the cell mitosis is the first vital process affected by this limitation. That the nuclei remain normal in size for a short time after the cessation of mitosis shows that anabolism is still in progress and may proceed with less food than the process of division. Elongation of cells continues while nuclei are decreasing in size which shows that tissue-building continues after the balance between anabolism and catabolism has been lost. Respiration, doubtless, continues until the ectoplasts are broken down and the loss of turgor results, which is designated by Blackman as the death-point.

Summary

1. The average increase in length of normally starved tips varies directly with the initial lengths, and the percentage increase in length varies inversely with the initial length.

2. Ether, in the given solutions, hastens the process of mitosis, but lessens the growth in length of the root. This agrees with Gerassimow's view, namely, that ether causes the cell to become physiologically weaker.

3. The greater length of seedlings grown in calcium-magnesium solutions shows that these elements in these proportions have a tonic effect and thus the cells are made physiologically stronger.

4. Cells from ether solutions are smaller in size than those grown under normal conditions, proving an exception to the rule of Eugen Schultz, namely, that increase and decrease in the size of an organism is not a function of cell size.

5. A decrease in the number and size of the small nucleoli of the older cells from the ether solutions, precedes the decrease in size of the nuclei while the cells are elongating. This is an indication that both nuclei and nucleoli are used in tissue-building.

6. An indication that the action of ether upon the protoplasm is a chemical one is found in the continued mitosis within rudimentary lateral roots after division has ceased in the tip, where the ether first gained entrance.

7. Plasmolysis of the cells shows that exosmosis of albumen may take place under ether influence as stated by Lalaski.

8. That regeneration of albumen under ether influence, as found by Lalaski in wheat seedlings, does not take place in Vicia faba under the experimental conditions here given is shown by the reduced quantity of chromatin in the nuclei.

9. The normal features of the cells of the formative region with the depleted appearance of the older cells in the region of differentiation give evidence in support of Loew's views that calcium and magnesium used in the given proportions facilitates the translocation of food materials.

10. The variation in density of the nucleolus gives support to one phase of the argument of Waratore, namely, that the nucleolus serves as the metabolic center of the nucleus.

Citations

1. Buscalioni, Giorn della R. Acad. di Med. Torino, 1892; cit. by Nathansohn Jahrbücher für Wissenschaftliche Botanik, Bd. 35, 1900, p. 48.
2. Gerassimow, Über de Grösse des Zellkerns. Botanisches Centralblatt. Beihefte, 1905, Vol. XVIII, Part 1, p. 45.
3. Hof. Histologische Studien an Vegetationspunkten.
4. Loew. Physiological role of Mineral Nutrients. Bulletin No. 1, Bureau Plat Industry.
5. Nathansohn. Physiologische Untersuchungen über amitotische Kerntheilung. Jahrbücher für Wissenschaftliche Botanik, Bd. 35, 1900, p. 48.
6. Pfeffer, Physiology of Plants. Vol. II, p. 267.
7. Rosen, Beitrage zur Kentniss der Pflanzenzellen. Beitrage zur Biologie der Pflanzen, Bd. 7, 1896, p. 225.
8. Eugen Schultz, Über Reductionen, I, Über Hungererscheinungen bei Planaria lactea. Archiv für Entwicklungsmechanik der Organismen. Vol. XVIII, 1904.
9. Zalaski. Zur Aetherwirkung auf die Stoffumwandlung in den Pflanzen. Berichte der Deutschen Botanischen Gesellschaft, Bd. 18. 1900. p. 292.
10. Paratore, Malpighia XV, 1902, p. 178-187.
11. Blackman, Optima and Limiting Factors, Annals of Botany, 1905. New Phytoligist, 1904, p. 33.
12. May, The Relation of Lime and Magnesia to Plant Growth, Bulletin No.1, 1901. Bureau of Plant Industry.